

Serial No.: 10/030,330

Confirmation No.: 4105

Filed: October 19, 2001

For: A POLYPEPTIDE HAVING AMIDOLYTIC ACTIVITY FOR A SERPIN

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**Amendments to the Claims**

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

1. **(Currently Amended)** An isolated oral bacterial polypeptide which has amidolytic activity for cleavage of a nondenatured human  $\alpha_1$ -proteinase inhibitor at a reactive site loop region of the inhibitor,

wherein the isolated polypeptide is encoded by the nucleic acid of claim 23 ~~comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.~~

2. **(Original)** The isolated polypeptide of claim 1 wherein the polypeptide has amidolytic activity in a solution comprising about 1 mM to about 500 mM Tris, about 500  $\mu$ M to about 100 mM cysteine maintained at a pH of about 7 to about 8.

3. **(Original)** The isolated polypeptide of claim 1 which is isolated from *Porphyromonas gingivalis*.

4. **(Original)** The isolated polypeptide of claim 1 which is a cysteine proteinase.

5. **(Original)** The isolated polypeptide of claim 1 which has a molecular weight of about 70 kD to about 80 kD as determined by gel filtration.

6. **(Original)** The isolated polypeptide of claim 1 which cleaves the reactive site loop region of the inhibitor represented by SEQ ID NO: 4 between glutamine and alanine.

7. **(Original)** The isolated polypeptide of claim 6 which cleaves the reactive site loop region of the inhibitor represented by SEQ ID NO: 4 between phenylalanine and leucine.

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8. **(Currently Amended)** An isolated polypeptide which is an oral bacterial cysteine proteinase and has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin,

wherein the isolated polypeptide is encoded by the nucleic acid of claim 23 ~~comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.~~

9. **(Original)** The isolated polypeptide of claim 8 wherein the polypeptide has amidolytic activity in a solution comprising about 50 mM Tris, about 20 mM cysteine maintained at a pH of about 7.4 at 37°C.

10. **(Original)** The isolated polypeptide of claim 8 which is isolated from *Porphyromonas gingivalis*.

11-15. **(Canceled)**

16. **(Currently Amended)** The isolated polypeptide of claim 8 ~~[[11]]~~, wherein the polypeptide further nonspecifically cleaves the serpin in a denaturing environment.

17. **(Canceled)**

18. **(Previously Presented)** An isolated polypeptide comprising an amino acid sequence represented by SEQ ID NO: 1.

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19. **(Currently Amended)** An isolated polypeptide encoded by the nucleic acid of claim 23 comprising an amino acid sequence having a percentage amino acid identity of greater than 37% to that amino acid 148 to amino acid 843 of SEQ ID NO: 1,

wherein the polypeptide has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin.

20. **(Previously Presented)** An isolated polypeptide comprising an amino acid sequence represented by amino acid 148 to amino acid 843 of SEQ ID NO: 1.

21-22. **(Canceled)**

23. **(Previously Presented)** An isolated nucleic acid encoding a polypeptide which has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin,

wherein a complement of the nucleic acid hybridizes to SEQ ID NO: 2 under hybridization conditions of 0.5 M phosphate buffer, pH 7.2, 7% SDS, 10 mM EDTA, at 68°C, followed by three 20 minute washes in 2x SSC, 0.1% SDS, at 65°C.

24. **(Previously Presented)** An isolated nucleic acid fragment encoding a polypeptide which is isolated from *Porphyromonas gingivalis* and has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin, wherein the nucleic acid has a nucleotide sequence comprising SEQ ID NO: 2.

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25. **(Currently Amended)** An isolated nucleic acid encoding a polypeptide which has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin,

wherein the encoded polypeptide comprises an amino acid sequence comprising having a percentage amino acid identity of greater than 37% to amino acid 148 to amino acid 843 of SEQ ID NO: 1.

26. **(Currently Amended)** An isolated nucleic acid encoding a polypeptide which has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin,

wherein the encoded polypeptide comprises an amino acid sequence comprising having a percentage amino acid identity of greater than 37% to amino acid 148 to amino acid 629 [[843]] of SEQ ID NO: 1, and

~~wherein a complement of the nucleic acid hybridizes to SEQ ID NO: 2 under hybridization conditions of 0.5 M phosphate buffer, pH 7.2, 7% SDS, 10 mM EDTA, at 68°C, followed by three 20 minute washes in 2x SSC, 0.1% SDS, at 65°C.~~

27. **(Currently Amended)** A method for identifying an inhibitor of a polypeptide which has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of a serpin comprising isolating an agent that inhibits the amidolytic activity of the polypeptide by incubating the polypeptide encoded by the nucleic acid of claim 23 with the agent under conditions that promote amidolytic activity of the polypeptide and determining if the amidolytic activity of the polypeptide is reduced relative to the amidolytic activity of the polypeptide in the absence of the agent, whereby the inhibitor is selected

~~wherein the polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.~~

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28. **(Original)** The method of claim 27 wherein the polypeptide is isolated from *Porphyromonas gingivalis*.

29. **(Currently Amended)** An immunogenic composition comprising a polypeptide which has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of a serpin that is capable of eliciting antibodies in an animal,

wherein the polypeptide is encoded by the nucleic acid of claim 23 ~~comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.~~

30. **(Canceled)**

31. **(Previously Presented)** The isolated nucleic acid of claim 23 wherein the polypeptide is isolated from *Porphyromonas gingivalis*.

32. **(Previously Presented)** The isolated nucleic acid of claim 25 wherein the polypeptide is isolated from *Porphyromonas gingivalis*.

33. **(Withdrawn - Currently Amended)** A method of inhibiting the amidolytic activity of a polypeptide comprising combining the polypeptide with an agent selected from the group consisting of dichloroisocoumarin, diisopropylfluorophosphate, leupeptin, tosyl-L-lysine chloromethyl ketone, Phe-Pro-Arg chloromethyl ketone, Z-Phe-Lys benzoyloxy methyl ketone, idoacetamide and L-trans-epoxysuccinyl-leucylamide-(4-guanidino)-butane (E-64),

wherein the polypeptide has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin, and

wherein the polypeptide is encoded by the nucleic acid of claim 23 ~~comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.~~

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34. **(Withdrawn - Currently Amended)** A method of inhibiting the amidolytic activity of a polypeptide comprising:

identifying an inhibitor that inhibits the amidolytic activity of the polypeptide by incubating the polypeptide encoded by the nucleic acid of claim 23 with the agent under conditions that promote amidolytic activity of the polypeptide and determining if the amidolytic activity of the polypeptide is reduced relative to the amidolytic activity of the polypeptide in the absence of the agent; and

combining the polypeptide with the agent,

wherein the polypeptide has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin, and

~~wherein the polypeptide comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.~~

35. **(Withdrawn - Currently Amended)** A kit for inhibiting the amidolytic activity of a polypeptide comprising:

an agent selected from the group consisting of dichloroisocoumarin, diisopropylfluorophosphate, leupeptin, tosyl-L-lysine chloromethyl ketone, Phe-Pro-Arg chloromethyl ketone, Z-Phe-Lys benzoyloxy methyl ketone, idoacetamide and L-trans-epoxysuccinyl-leucylamide-(4-guanidino)-butane (E-64); and

instructions for combining the agent with a polypeptide,

wherein the polypeptide has amidolytic activity for cleavage of a nondenatured serpin at a reactive site loop region of the serpin, and

wherein the polypeptide is encoded by the nucleic acid of claim 23 ~~comprises an amino acid sequence having a percentage amino acid identity of greater than 37% to that of SEQ ID NO: 1.~~

36. **(Previously Presented)** An isolated nucleic acid encoding a polypeptide comprising SEQ ID NO:1.

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37. **(Previously Presented)** An isolated nucleic acid encoding a polypeptide consisting of SEQ ID NO:1.

38. **(Previously Presented)** An isolated polypeptide comprising an amino acid sequence represented by amino acid 148 to amino acid 629 of SEQ ID NO:1.

39. **(New)** A vector comprising the nucleic acid of claim 23.

40. **(New)** The vector of claim 39 wherein the vector is an expression vector or a cloning vector.

41. **(New)** The vector of claim 39 wherein the vector is selected from the group consisting of plasmid vectors, viral vectors, cosmid vectors, and artificial chromosome vectors.